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*Published in:*
76th Cornell Nutrition Conference For Feed Manufacturers

*Publication date:*
2014

*Citation for published version (APA):*
Marcelo, G-B., Foskolos, A., Ross, D. A., & Van Amburgh, M. E. (2014). The effect of protein intestinal digestibility on nitrogen efficiency and performance in high producing dairy cattle. In *76th Cornell Nutrition Conference For Feed Manufacturers*

# BALANCING FOR INTESTINAL NITROGEN INDIGESTIBILITY IN HIGH PRODUCING LACTATING CATTLE: ONE STEP CLOSER TO FEEDING A COW LIKE A PIG?

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## INTRODUCTION

This paper is a continuation of the work by Ross et al. (2013), which described the development of an assay to estimate the intestinal indigestibility of nitrogen in cattle. Current cattle diet formulation models rely on library estimates of intestinal digestibility of proteins and carbohydrates to predict metabolizable energy (ME) and protein (MP) supply (NRC, 2001; Fox et al., 2004; Tylutki et al., 2008). As models become more accurate and precise in the prediction of nutrient supply and nutrient balance, there is a greater need to evaluate and be able to adapt the inputs currently used as static library values. Although CP is not a functional dietary nutrient for cattle, many diets are still formulated on this metric, creating confusion due to inadequate information provided by the value, especially with regard to MP supply and amino acid availability. As diets are formulated closer to the MP requirements of cattle and subsequently lower in CP, accurate estimates of intestinal digestibility (ID) or indigestibility of protein and amino acids are increasingly important to ensure an adequate supply of those nutrients. Use of outdated feed library values to all feeding conditions can lead to under- and over-estimations of MP and amino acid supply, resulting in variation from expected production.

Since the inception of the Cornell Net Carbohydrate and Protein System (Fox et al., 2004; Tylutki et al., 2008), the detergent system of fractionation has been applied to both the carbohydrate and protein components of feeds (Sniffen et al., 1992). More recent work suggests this approach, especially for feeds not containing NDF, might not be appropriate to accurately characterize how protein is partitioned and digests in the rumen and post-ruminally. Several approaches have been developed to predict the intestinal digestibility of protein in feeds and are a departure from the detergent system of feed chemical composition (Calsamiglia and Stern, 1995; Ross et al., 2013). The study described in this paper was conducted by formulating two different diets in high producing cattle using two different blood meals with different predicted intestinal protein indigestibility to test the accuracy and precision of both the assay (Ross et al., 2013) and our ability to apply those values in the CNCPS for diet formulation. The assay was developed to predict N indigestibility, and will be described in that manner throughout the paper.

## MATERIALS AND METHODS

### *Treatments, Animals and Experimental Design*

Treatments were established from a quantity of two blood meals secured through the marketplace that would allow an inclusion level of approximately 1 kg per head per day for the entire experimental period. The two blood meals were analyzed for unavailable N (uN) prior to the start of the study using the in-vitro assay described by Ross et al. (2013). Briefly, 0.5g of sample are placed into a 125ml Erlenmeyer flask. 40ml of rumen buffer and 10ml of rumen fluid are added to each flask. Flasks are incubated in a water bath at 39°C for 16h under continuous $CO_2$. Samples are then acidified with 3M HCL to bring the pH down to 2. Samples are incubated on a shaking bath for one hour after the addition of 2ml of pepsin and pH 2 HCl. Samples are then neutralized with 2ml of 2M NaOH to stop the pepsin reaction. An enzyme mix containing trypsin, chymotrypsin, lipase and amylase is added to the flask and incubated for 24h in the shaking bath at 39°C. Samples are then filtered with a 1.5 µm glass filter and boiling water. Nitrogen content of the residue is determined by Kjeldahl and expressed as a % of total N in the sample. The blood meals are characterized by their predicted intestinal N indigestibility (INID) since that is the outcome of the assay. The predicted INID of the low (LOW treatment) INID blood meal was 9%, whereas that of the other treatment (HIGH) was 33.8%. Thus, the two dietary treatments were established by inclusion of these blood meals in two different diets on an iso-N basis. The rest of the diets were formulated to be identical. The low uN blood meal was 15.04% N and the higher uN blood meal was 14.6% N, thus at approximately 1 kg inclusion level, the maximum difference in intestinal N availability was 38.5g N g. The composition of the two diets fed to cattle is in Table 1.

Due to changes in milk yield in both treatments due to stage of lactation, the protein content of both diets was adjusted down at approximately 5 weeks of treatment by reducing the canola meal inclusion level by 50% to be more consistent with the ME allowable milk and to maintain the N supply to a level the cattle should remain sensitive to the treatment differences in N availability created by the inclusion of the two different blood meals.

Ninety-six multiparous cows (726 ± 14.2 kg BW; 147 ± 64 DIM) and thirty-two primiparous cows (607 kg ± 29.5kg BW; 97 ± 20 DIM) were distributed by DIM and BW into 8 pens of 16 cows (12 multiparous and 4 primiparous). Pens were stratified into four levels of milk production, and each stratum randomly allocated to treatments. Diets were formulated using Cornell Net Carbohydrate and Protein System (CNCPS v6.1; Van Amburgh et al., 2013) using the chemical composition of the ingredients used in the experimental diets.

The lactation trial consisted of a two week adaptation period, one week covariate period and 9 week experimental period, between March 30 and June 21 2014 at Cornell University Ruminant Center (Harford, NY). All cows were fed the LOW diet during adaptation and covariate periods. Cows were housed in pens under a four row barn design with one bed and more than one headlock per cow and free access to water. All cows received rBST (Posilac, Elanco Animal Health, Indianapolis, IN) on a 14 day schedule throughout the length of the trial.

**Table 1.** The ingredient content and chemical composition of two diets containing blood meals with Low and High indigestible intestinal N digestibility.

| | Treatment | |
|---|---|---|
| Ingredient, % DM | LOW uN | HIGH uN |
| Alfalfa haylage | 11.5 | 11.5 |
| BMR corn silage | 49.3 | 49.3 |
| Bakery | 1.8 | 1.8 |
| Blood meal High | 3.7 | --- |
| Blood meal Low | --- | 4.0 |
| Canola meal | 3.0 | 3.0 |
| Corn grain | 16.1 | 16.1 |
| Energy Booster 100 | 1.8 | 1.8 |
| Molasses | 1.8 | 1.8 |
| Smartamine M | 0.1 | 0.1 |
| Sodium bicarbonate | 0.6 | 0.5 |
| Soybean hulls | 4.6 | 4.5 |
| Urea | 0.2 | 0.2 |
| Wheat midds | 4.6 | 4.5 |
| Min/vit mix | 1.0 | 1.0 |
| | | |
| *Chemical composition* | | |
| DM, % as fed | 50.0 | 50.5 |
| CP, % DM | 15.2 | 15.2 |
| NDF, % DM | 31.9 | 32.3 |
| ADF, % DM | 21.3 | 20.5 |
| Ether extract, % DM | 4.3 | 3.9 |
| Starch, % DM | 30.4 | 31.2 |
| Sugar, % DM | 3.6 | 3.3 |
| Ca, % DM | 0.65 | 0.60 |
| P, % DM | 0.43 | 0.43 |
| ME[1], Mcal/kg DM | 1.8 | 1.7 |
| Lys:Met[1], % MP | 3.21 | 3.19 |

LOW: low uN diet, HIGH: high uN diet. [1]CNCPS predicted

Cattle were fed once per day for approximately 5% refusal and milked 3 times per day at 6:00, 14:00 and 22:00 and data from all milkings was recorded using Alpro herd management system (DeLaval International AB, SG). Individual milk samples were

collected weekly during three consecutive milkings, and preserved with 2-bromo-2-nitropane-1, 3-diol at 4°C until analyzed. Milk yield was expressed as 3.5% energy corrected milk (ECM) according to the equation of Tyrell and Reid (1965): ECM (kg) = (12.82 * kg fat) + (7.13 * kg protein) + (0.0323 * kg milk).

Cattle were weighed once per week using a walk scale XR3000 (Tru-test, TX) after the morning milking. Further, BCS on a scale of 1 to 5 was determined every two weeks by the same two evaluators. An average of the two evaluators was used as the mean BCS.

*Statistical analyses*

Data was analyzed using the following mixed effects model (JMPv.11 SAS Institute, Inc., Cary, NC):

$Yijk = Ti + Wj + TWij + Bl + ck(P) + E$

where,

Yijlk is the dependent, continuous variable,

Ti is the fixed effect of the ith treatment (i=1, 2),

Wj is the fixed effect of the jth week (j=1, …, 9),

TWij is the fixed effect of the interaction between the ith treatment and the jth week,

Bl is the covariate measurement for the lth cow or the lth pen,

ck is the random effect of the lth cow nested within pen or lth pen depending on the variable tested,

eijlk is the residual error.

The statistical unit for milk yield, milk components, BW and BCS was the random variable cow nested within pen, while for DM and N intake was the random variable pen.

The balanced design (equal number of cows per pen and equal number of pens per treatment) allowed using the animal as the error term for the analysis of milk, BW and BCS data plus allowed excluding the random variable pen without overestimating the error degrees of freedom of the model (St Pierre, 2007). Regression analysis was used to calculate weight gain for individual cows accounting for the need to include week in the statistical model for that variable. Overall treatment differences were evaluated using least square means. Significance was declared at P-values <0.05.

## RESULTS AND DISCUSSION

*Animal Performance*

Overall DMI and N intake for the treatments were similar and milk yield was significantly different for cattle fed the two treatments (Table 2). Milk yield was 1.6 kg/d lower for cattle fed the HIGH uN diet and energy corrected milk (ECM) was 1.9 kg/d lower on the same diet. Further, cattle fed the HIGH uN diet had significantly lower MUN levels that cattle fed the LOW uN diet (Table 2). From this information, it is apparent that the cattle fed the different blood meals had significantly different MP supply, consistent with the predicted values from the uN assay. The predicted difference described earlier (38.5 g N) is equal to approximately 240 g MP, about the amount required to produce 5 kg of milk under the conditions of this study.

Table 2. Effect of N availability on intake, milk production, milk composition and body weight gain of dairy cows fed diets with low and high unavailable N

| | Treatment | | | |
|---|---|---|---|---|
| Item[1] | LOW uN | HIGH uN | SEM | P-value |
| DMI, kg | 27.4 | 27.1 | 0.61 | 0.75 |
| N Intake, kg DM | 671.1 | 664.4 | 14.8 | 0.77 |
| *Milk production* | | | | |
| Milk, kg | 42.0 | 40.4 | 0.31 | <0.01 |
| ECM, kg | 41.9 | 40.0 | 0.32 | <0.01 |
| Fat, kg | 1.51 | 1.42 | 0.02 | <0.01 |
| Protein, kg | 1.26 | 1.23 | 0.01 | 0.03 |
| *Milk composition* | | | | |
| Fat, % | 3.6 | 3.5 | 0.03 | <0.03 |
| Protein, % | 3.03 | 3.06 | 0.02 | 0.20 |
| Lactose, % | 4.90 | 4.86 | 0.02 | 0.18 |
| MUN, mg/dl | 9.4 | 8.0 | 0.18 | <0.01 |
| SCC (log1000/ml) | 3.9 | 4.0 | 0.05 | 0.13 |
| *BW and BCS* | | | | |
| BWinitial, kg | 684.1 | 692.1 | 10.1 | 0.58 |
| BWchange, kg | 34.7 | 29.7 | 2.25 | 0.12 |
| BCSchange, (1-5) | 0.20 | 0.16 | 0.03 | 0.29 |
| *Efficiency* | | | | |
| Feed efficiency[2] | 1.56 | 1.50 | 0.03 | 0.34 |
| Milk N efficiency[3] | 30.0 | 29.7 | 0.70 | 0.76 |

[1] DMI: dry matter intake, ECM: energy corrected milk yield (Tyrrell and Reid, 1965),
[2] calculated as kg milk / kg DMI
[3] calculated as milk N/N intake*100

However, the observed difference on an ECM basis was 1.9 kg, thus the difference between the absolute levels measured in the assay and the observed ECM yield are either due to differences in digestibility within the cow, the amount of the blood meal arriving at the small intestine or the amount of nutrients partitioned to body reserves, or a combination of all of those factors. Although the change in BW and BCS were not significant, the changes are still biologically relevant given the partitioning of nutrients to reserves and away from milk.

To evaluate the outcome of the study, CNCPS v6.1 with the updated feed library rates and pool sizes was used to evaluate the predictions. The chemical composition of the feeds used in the study was inputted into the model. To evaluate the assay within the structure of the model and against the study data, the blood meal values for the uN and ADIN were the only values changed. For the two blood meals, the uN values were inputted in place of the ADIN value, and intestinal digestibility left at zero. Further, the intestinal digestibility of the NDIN value were set to 100% although after being analyzed for aNDFom, the blood meals do not contain any ND residue, so that pool is zero. With this approach, all of the protein in blood meals is in the A2, B1 and C fractions.

The current intestinal digestibility of the NDIN fraction for all feeds is 80% and it appears that the assay of Ross et al. (2013) captures that portion of the indigestible protein, therefore by difference; the remaining fractions should be set at 100% digestibility. Thus, with continued testing and implementation of the uN assay for all feeds, the NDIN fraction ID will be set to 100% because it appears that in NDF containing feeds, the uN assay spans both the ADIN and NDIN fractions.

For the cattle inputs, the expected BW change based on the target growth approach was used and the BCS change was also inputted over the period of the study (9 wks), thus this accounted for the distribution of nutrients to other productive uses and not just milk output. With all of the inputs accounted for, the prediction of ME and MP allowable milk with the uN assay information is in Table 3.

In the CNCPS evaluation in Table 3, it is apparent that the feed chemistry described through the detergent system is not appropriate to allow the model to predict the most limiting nutrient in this comparison using blood meal as the treatment. When the uN data are used to describe the chemistry of the blood meals, the model provides an acceptable and realistic prediction of the most limiting nutrient. It is also important to recognize that an accurate and complete description of the animal characteristics was important to make this evaluation and in the absence of that information, the model would predict over 4 kg of MP allowable milk difference. The sensitivity of the model predictions to complete and accurate animal characterization cannot be overstated and

helps explain why literature data to evaluate the model rarely allows for robust predictions of most limiting nutrients due the lack of complete information.

Table 3. The actual and energy corrected milk and the metabolizable energy (ME) and protein (MP) allowable milk for both treatments predicted by the CNCPS using the assay data of Ross et al., (2013) to estimate intestinal digestibility of blood meal, or using the original fractionation approach using acid detergent insoluble nitrogen as the unavailable fraction.

| | Treatment | |
|---|---|---|
| | Low uN | High uN |
| Actual milk, kg | 42.0 | 40.4 |
| Energy corrected milk, kg | 41.9 | 40.0 |
| uN assay inputs | | |
| ME allowable milk, kg | 45.0 | 46.0 |
| MP allowable milk, kg | 42.6 | 39.3 |
| Using NDIN and ADIN | | |
| MP allowable milk, kg | 44.9 | 44.6 |

In summary, the uN assay appears to provide protein indigestibility predictions that are consistent with cattle responses and serves as a platform for modifying the approach to predict digestibility within the CNCPS and will improve the model's ability to identify the most limiting nutrient. The data also demonstrate we are ready to move beyond the detergent system of fractionation for protein and move to a system that fractionates proteins based on solubility and indigestibility. This approach should allow us to develop a prediction model to more effectively estimate rates of protein degradation because we now have what appears to be a more robust method to predict the indigestible protein pool, consistent with the approach for NDF (Raffrenato et al., 2009) and this fraction is important for accurate calculations of the rate of digestion of the available protein fraction.

We gratefully acknowledge the staff at the Cornell University Ruminant Center (CURC) Dairy, Dennis Stucker and Perdue Agsolutions, Brian Sloan and Addiseo and Chip Hyde at CNY Feeds for support of this study.

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